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**Highlights**

**Differentiation**

**Time to change jobs?**

At present, 150 million people worldwide are diabetic and, in *Current Biology*, Slack and colleagues now provide a possible alternative treatment for insulin-dependent diabetes. In embryonic development, the liver and pancreas are made from adjacent regions of endoderm, and they’ve managed to change the job of differentiating or differentiated liver cells by converting them into pancreatic cells.

The transcription factor Pdx1 probably determines the difference between the pancreas and the liver but, in previous studies that over-expressed Pdx1 in various sites, no ectopic pancreas was produced. This led Slack and co-workers to suggest that suitable Pdx1 binding partners might be missing in the tissues studied, so they designed a modified active form of Pdx1 that does not require such partners.

The modified Pdx1 consisted of the VP16 activation domain from *Herpes simplex* virus fused to the carboxyl terminus of Xlhbox8 (the *Xenopus laevis* Pdx1 homologue). The mouse transthyretin (*TTR*) promoter — which directs expression to the liver — was used to control *Xlhbox8–VP16* expression and, to simplify visualization, the transgene included green fluorescent protein (GFP) under the control of the elastase promoter, which marks pancreatic differentiation.

*Xenopus* tadpoles containing the *TTR–Xlhbox8–VP16:elastase–GFP* transgene initially developed normally but, after 5 days, Slack and colleagues could see fluorescence in an area normally occupied by liver. In this area, they detected the presence of insulin and glucagon messenger RNAs (products of pancreatic endocrine cells) and of amylase mRNA (a product of pancreatic exocrine cells), which indicates the presence of differentiated pancreas that contains both exocrine and endocrine tissues.

When the authors looked at the expression of endogenous *TTR* mRNA in the transgenic tadpoles, they found that this liver-specific marker is quickly downregulated in the ectopic pancreas. Furthermore, they showed that the liver is already differentiating before the expression of the transgene is induced, which indicates that this conversion is ‘transdifferentiation’, rather than a change of developmental pathway in the embryo.

So, could liver cells be made to change jobs in humans? It seems the answer is yes, because when Slack and co-workers transfected the transgene into the human liver cell line HepG2, they found that most of the differentiated liver cells that received the construct converted into pancreatic cell types.

Expression of *Xlhbox8–VP16* is only transient in the liver, because *TTR* expression is downregulated as the conversion to pancreas occurs. However, once established, the ectopic pancreas persists. This procedure, which requires no permanent genetic change, could therefore represent an important discovery in the hunt for new treatments for insulin-dependent diabetes.

Rachel Smallridge

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**References and links**

**Original Research Paper**  

**Further Reading**  
Double trouble

p53 can kill cells by transcription-dependent means, and it can enhance this effect by inducing death by a transcription-independent mitochondrial pathway. Reporting in Molecular Cell, Ute Moll’s group show that p53 does this by translocating to mitochondria, and inducing permeabilization of the outer mitochondrial membrane (OMM) and the release of cytochrome c.

Previously, the authors saw that a fraction of p53 translocates rapidly to mitochondria after apoptotic stimuli (DNA damage and hypoxia) in malignant and immortal cells. So they γ-irradiated mouse thymocytes to see if this also occurs in primary cells. Within 1 h of treatment, p53 moved to the mitochondria.

By targeting p53 directly to mitochondria of p53-null cells and using several sensitive transciptional assays, Moll’s group could discount the involvement of p53’s transactivation activity during the ensuing apoptosis.

Because p53 translocates to the surface of mitochondria, the authors looked for a link with the anti-apoptotic mediators Bcl2 and BclXL, which are anchored constitutively at the OMM, and found that p53 formed a specific complex with Bcl2 and BclXL.

Death-stimulus-induced mitochondrial translocation of p53 precedes the release of cytoochrome c, and the authors next showed that purified p53 could release cytochrome c from mitochondria in a dose-dependent manner. It does this by forming inhibitory complexes with BclXL (which stabilizes the OMM) and by conformationally changing, and thereby indirectly activating, Bak and Bax proteins (which are the ultimate effectors of cytochrome c release).

The authors propose that p53, by moving rapidly to the mitochondria, effectively ‘jump-starts’ and amplifies its slower-starting transcription-dependent effect on apoptosis. From a cancer aspect, some p53 mutants can’t interact with BclXL to promote cytochrome c release, so mutations that are selected for during human tumorigenesis might represent ‘double-hit’ mutations that inhibit both the transcriptional and mitochondrial apoptotic activities of p53.

Rachel Smallridge

A deadly gang

When attacking prey, animals often work together as a deadly gang and, on a smaller scale, it’s teamwork that is crucial for a successful attack by the three subunits of the Bacillus anthracis anthrax toxin. The first subunit, the 83-kDa form of protective antigen (PA83), binds to the host cell’s anthrax toxin receptor (ATR), which induces a cleavage reaction that produces PA63. PA63 then oligomerizes to form (PA63)7, binds to the other two subunits lethal factor (LF) and oedema factor (EF) and facilitates their entry to the cytoplasm, where these factors exert their toxic effects. The details of this initial entry process have been unclear, but now, in The Journal of Cell Biology, van der Goot and colleagues provide new insights.

The authors first showed that, whereas PA83 associates with detergent-soluble domains of the plasma membrane, PA63 associates with detergent-resistant membranes (DRMs or lipid rafts) or, more specifically, with “noncaveolar cholesterol and sphingolipid-rich domains of the plasma membrane”. They then showed that free ATR is not raft associated, so it seems that PA63 forces ATR into rafts. Furthermore, they found that LF, which binds (PA63), and not PA63, associates with DRMs in PA63-treated cells, which indicates that (PA63), is predominant in rafts.

So, is it ATR clustering, which is a result of PA63 heptamerization, that causes the raft association? It seems so, because when van der Goot and co-workers used an alternative method to cluster ATR — that is, they labelled ATR with PA83 (which cannot heptamerize) and then added monoclonal or polyclonal antibodies against PA83 — they found that clustered PA83 was mainly present in DRMs, in contrast to non-clustered PA83. In addition, they showed that PA clustering was essential and sufficient to promote its internalization.

Having ruled out a caveolin-mediated entry pathway, van der Goot and colleagues showed that PA enters cells by clathrin-mediated endocytosis (caveolin- and clathrin-mediated uptake) and Eps15 (which is required specifically for clathrin-mediated endocytosis) had inhibitory effects on PA internalization.

The physiological role of ATR is not clear, but this work has shown that “its trafficking properties, i.e., slow endocytosis as a monomer and rapid clathrin-mediated uptake on clustering, make it an ideal anthrax toxin receptor”. In addition, this study has highlighted the potential importance of lipid rafts as therapeutic targets for drugs against anthrax.

Rachel Smallridge

References and links

WEB SITE Ute Moll’s laboratory: http://www.path.sunysb.edu/faculty/umoll/default.htm

WEB SITE Gisou van der Goot’s laboratory: http://www.medicine.unige.ch/recherche/groupes/f/fondamentale/
Down a hairpin

Short hairpin RNAs (shRNAs) — synthetic molecules that are modelled on small, non-coding microRNA molecules with a ‘hairpin’ secondary structure — can silence gene expression by RNA interference (RNAi), much as small interfering RNAs (siRNAs) do. As now reported in Nature Structural Biology, Thomas Rosenquist’s group, in collaboration with Greg Hannon’s group, explored whether germline transmission of shRNA constructs was feasible in mammals, as this would enable stable, long-term silencing of gene expression.

The initial attempt of Rosenquist and colleagues to achieve germline transmission using standard transgenics methods, in which linearized constructs were injected into pronuclei to create transgenic founder animals, was unsuccessful. Choosing Neill (which encodes a DNA N-glycosylase that initiates base-excision repair) as the target gene, they turned to a different approach — based on the use of embryonic stem (ES) cells. The authors created a single shRNA expression construct against Neill, which was introduced into mouse ES cells by electroporation. Stable ES cell lines showed ~80% reduction of Neill protein, which correlated with a similar reduction in mRNA levels, and the cells were approximately twofold more sensitive to ionizing radiation — consistent with the role of Neill in DNA repair.

To generate transgenic animals, the authors injected cells from two independent ES cell lines into blastocysts. The chimeric mice that contained a high percentage of ES-derived cells were outcrossed and germline transgenic mice in which a stable, long-term silencing of gene expression. The authors concluded, therefore, that shRNAs can be used to create germline transgenic mice in which a target gene is silenced by RNAi. These findings now open the door for tissue-specific, inducible and reversible suppression of gene expression in mice.

Ariane Heinrichs

References and links


HIGHLIGHTS

Two become one

Take two pathways — Cdc42–Par6–protein kinase Cζ (PKCζ), and glycogen synthase kinase-3β (GSK-3β)—β-catenin–adenomatous polyposis coli (Apc) — that have previously, but independently, been implicated in the control of cell polarity and what have you got? A single mechanism to spatially control cell polarity, report Alan Hall and Sandrine Etienne-Manneville in Nature.

What lay downstream of Par6–PKCζ in cell-polarity control was unknown, so Hall and Etienne-Manneville studied GSK-3β phosphorylation. Using a scratch-induced cell migration assay, they noticed an increase in the levels of GSK-3β phosphorylated at serine 9 soon after scratching. Anti-phospho-GSK-3 (Ser9) staining occurred predominately at the leading edge of migrating cells, colocalizing with Cdc42, where Par6 and PKCζ have previously been observed. They also showed that GSK-3β and PKCζ are in a complex, which dissociates during scratch-induced migration, and that the same complex might also contain Par6. As phosphorylated GSK-3β couldn’t be detected in PKCζ precipitates, phosphorylation probably causes GSK-3β to dissociate from the complex.

Inhibiting Cdc42 or PKCζ prevented GSK-3β phosphorylation, whereas transfection of Par6 or PKCζ induced GSK-3β phosphorylation. Phosphorylation of GSK-3β on Ser9 inhibits its catalytic activity, and when the authors microinjected a non-phosphorylatable, constitutively active mutant of GSK-3β (GSK-3 59A) into leading-edge cells, they saw that it blocked centrosome reorientation, which is a measure of polarity.

As in the Wnt pathway, inactivation of GSK-3β results in the accumulation of β-catenin, but during astrocyte migration, β-catenin accumulated at the leading edge and not in the nucleus. Although β-catenin is not required for polarity, Apc, another target of GSK-3β in the Wnt pathway, is. Two to four hours after scratch-induced migration, in a Cdc42−, PKCζ− and phospho-Ser9-GSK-3β-dependent manner, Apc associated with the plus ends of microtubules at the leading edge. Apc binds to microtubules directly or indirectly through the microtubule-binding protein EB1, but EB1 localization at microtubules was independent of Cdc42, PKCζ and GSK-3β. Deletion of the carboxy-terminal domain of Apc, which contains the microtubule- and EB1-binding sites, inhibited centrosome reorientation.

So the spatially restricted association of Apc with the plus ends of microtubules — crucial for establishing polarity — arises from the action of Cdc42 on Par6–PKCζ, which, in turn, results in GSK-3β phosphorylation and its inactivation. GSK-3β might affect several other microtubule-associated proteins, too.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Etienne-Manneville, S. & Hall, A. Cdc42 regulates GSK-3β- and adenomatous polyposis coli to control cell polarity. Nature 2003 Jan 29 (DOI: 10.1038/nature01428)

HIGHLIGHTS

STRUCTURE WATCH

A complex interaction

The cyclin-dependent kinase inhibitor Sic1 must be destroyed to allow cells to progress through the cell cycle, and this destruction occurs when Sic1 is phosphorylated at least on six of its nine Cdc4-phosphodegron (CPD) sites. This phosphorylated Sic1 is bound by the WD40 domain of the F-box protein Cdc4, which takes Sic1 to the SCF^Cdc4 (Skp1/Cullin/F-box protein) ubiquitin-ligase complex — a multisubunit E3 enzyme. Here, Sic1 is ubiquitylated, which targets it for proteolytic destruction. To clarify the basis of phospho-epitope recognition by Cdc4, and to further understand how E3 enzymes orientate their substrates, Sichler and colleagues now describe in Cell the 2.7 Å X-ray crystal structure of a Skp1–Cdc4 complex bound to a CPD phosphopeptide.

The structure showed that a core CPD motif — Leu-Leu-pThr-Pro — binds to an eight-bladed β-propeller WD40 domain in Cdc4, and it also clarified how F-box proteins present substrates for ubiquitin transfer. Furthermore, the authors found that the low-affinity binding of CPD motifs in Sic1 to Cdc4 reflects a structural incompatibility with the CPD-binding site in Cdc4. When they re-engineered Cdc4 to optimize Sic1 binding, lower phosphorylated forms of Sic1 were ubiquitylated. These data explain the phosphorylation threshold for Sic1 binding, and indicate “…an equilibrium binding mode between a single receptor site in Cdc4 and multiple low-affinity CPD sites in Sic1.”


An active Holliday

During genetic recombination, two homologous DNA molecules exchange strands to form a four-way DNA (Holliday) junction, and the subsequent action of junction-resolving enzymes determines the final genetic outcome. In the absence of Mg^2+, the centre of this junction is unstacked and open, and the four helical arms point towards the corners of a square. However, in the presence of Mg^2+, the junction folds to form one of two possible stacked X-structures, in which two DNA strands run straight through a pair of stacked helices and the other two are swapped between helical pairs. It is thought that transitions occur between these alternative stacking conformers, but no direct evidence has been presented to support this idea. Now, though, in Nature Structural Biology, Ha and colleagues describe the use of single-molecule methodology to detect these transitions in real time.

The authors found that the processes of conformer transition and branch migration both have the unstacked, open structure as the common intermediate, but that conformer transitions occur much faster than branch migration steps. Correlations have been observed between the dominant stacking conformation and the resolution of a Holliday junction, which indicates that DNA sequences can affect the outcome of genetic recombination by biasing the stacking conformation. The results of this study therefore indicate that “…sequence-dependent conformer bias can be fully manifested even in a fully branch-migratable junction and can determine the extent of genetic information exchange upon junction resolution”.


P53

Parc keeper

It’s a recurring theme in cell biology — in the complex and crowded environment of the cell, where you are is just as important as what you are. The tumour suppressor p53 is needed in the nucleus to prevent the growth of abnormal or damaged cells by activating genes involved in cell-cycle arrest and apoptosis. But p53 is not required during normal cell growth when there is no DNA damage and, consequently, large amounts of p53 can be found in the cytoplasm of unstressed cells. This, and the finding that many tumour cells have abnormally high levels of cytoplasmic p53, indicates that sequestration of p53 in the cytoplasm could represent a non-mutational control mechanism.

Now, in Cell, Gu and colleagues report the purification and characterization of a crucial factor in this process called Parc (p53-associated, parkin-like cytoplasmic protein). Parc is present as part of a large complex (~1 MDa) in the cytoplasm, and associates tightly with p53, with its amino terminus appearing to interact directly with the carboxyl terminus of p53. Sequence analysis showed that Parc contains several domains, including Ring–IBR–Ring and CCH (C-terminal Cullen homology domain) signature motifs, which are found in proteins associated with ubiquitin-ligase activity. But despite having an intrinsic ubiquitin-ligase activity, the authors found no direct evidence to suggest that Parc regulates p53 stability through ubiquitylation.

Gu and co-workers found that overexpression of Parc promoted the cytoplasmic sequestration of ectopic p53. Neuroblastoma cells were also found to express abnormally high cytoplasmic levels of Parc, and RNA-interference-mediated downregulation of Parc in these cells induced nuclear localization of p53 and p53-mediated apoptosis.

Taken together, these results suggest that Parc is a crucial regulator of the subcellular localization of p53. Whether Parc interacts with a protein called Mdm2, another ubiquitin ligase recently shown to be involved in the nuclear export of p53, will shed light on what seems to be yet another level of control of this remarkable protein.

Simon Frantz, Associate Editor, Nature Reviews Drug Discovery

References and links


Unpair to repair

Once they’ve been exposed to DNA damage, cells need to kick-start repair pathways post-haste. The ATM (ataxia-telangiectasia, mutated) kinase is known to be involved in this process — it initiates signal-transduction pathways after mammalian cells have been exposed to ionizing radiation (IR), which initiates strand breaks in DNA. Details of its exact role have been sketchy, but a report in Nature by Christopher Bakkenist and Michael Kastan now sheds new light on how ATM is activated.

The ATM protein belongs to the phosphoinositide-3-kinase family, but phosphorylates proteins rather than lipids. As transfected ATM is a phosphoprotein, it would make sense if its activity were modulated by post-translational modification. The authors showed that ATM is indeed phosphorylated at a specific residue, serine 1981, in 1981. Experiments with antibodies that recognize Ser1981 only when it is phosphorylated (anti-1981S-P) showed that this phosphorylation occurs in response to IR. Moreover, phosphorylation of transfected kinase-inactive ATM depended on the presence of kinase-active ATM, suggesting that ATM autophosphorylates in trans.

What are the functional implications of this phosphorylation? The authors next did biochemical studies of ATM’s domains and protein–protein interactions. They used glutathione-S-transferase (GST)-tagged proteins to show that the kinase and phosphorylation domains can bind to one another, and that the amino acids around Ser1981 are crucial for this interaction. This binding could theoretically occur within the same molecule (in cis) or between ATM molecules (in trans), so the authors tested whether ATM can form higher-order multimers by trying to covalently crosslink it using formaldehyde. They detected an ATM-containing complex that migrated more slowly than a denatured ATM monomer, was not seen if cells had been exposed to IR and was not recognized by the anti-1981S-P antibody.

Bakkenist and Kastan wondered whether ATM might normally exist as a higher-order complex that dissociates in response to IR through a process linked to intermolecular autophosphorylation at Ser1981. To test this, they transfected haemagglutinin (HA)-tagged ATM into 293T cells, along with wild-type-, kinase-inactive- or S1981A-Flag-tagged ATM. They then irradiated the cells and investigated which proteins bound to each other. Whereas HA-ATM could be immunoprecipitated by kinase-inactive- and S1981A-Flag-ATM after irradiation, it no longer bound wild-type-Flag-ATM. A picture emerges, then, in which ATM molecules are normally held in check in the cell by pairing up. When cells are irradiated, however, the partners phosphorylate one another and separate to repair the damaged DNA.

This model — though very elegant — doesn’t address how ATM detects the damage in the first place. But Bakkenist and Kastan might have the answer. They observed that doses of radiation as low as 0.5 Gy (which initiates very few strand breaks in DNA) can trigger the autophosphorylation of a surprisingly high fraction of the cellular ATM. This, say the authors, suggests “the introduction of DNA strand breaks must cause a change in the nucleus that can activate ATM at a distance from the break itself”. In other words, ATM does not need to bind to the actual break to initiate repair. Couple this with the fact that the strand breaks caused by IR alter the topological constraints on DNA, and you have the idea that changes in the structure of chromatin might be the signal that activates ATM. In support of this, drugs that alter the chromatin structures without generating DNA strand breaks were also able to activate ATM.

Alison Mitchell

References and links


Katrin Bussell
HIGHLIGHTS

APOTOPSIS

Follow the fly path

It all started two years ago, say the authors, with the discovery of p53 in Drosophila melanogaster. They reasoned that if Drosophila has p53, then maybe there are parallels between the way flies and mammals induce p53-dependent cell death. And it seems that they were right, as they now report in Genes and Development.

The authors in question — Arnold Levine and colleagues — started with a p53-dependent pathway that had been observed in flies but not in mammals. Drosophila p53 activates the transcription of a gene called Reaper, the product of which interacts with the inhibitor of apoptosis (IAP) protein DIAP and targets it for ubiquitin-mediated proteolysis. As DIAP’s role is to inhibit caspases, its destruction frees up caspases to proceed with an apoptotic response.

So could this happen in mammals? To test this, Levine and co-workers first looked for proteins that might interact with CIAP1 — the human homologue of DIAP1. They generated stable clonal HeLa cell lines that expressed a haemagglutinin-Flag-tagged CIAP1 protein, and then co-purified CIAP1 and its partners from HeLa cell lysates using M2-agarose beads, which recognize the Flag tag. In response to etoposide-induced p53-dependent apoptosis, the authors pulled down two low-molecular-weight proteins. But these weren’t CIAP1’s binding partners — they were fragments of CIAP1 itself.

Levine and colleagues concluded that CIAP1 is cleaved during p53-dependent apoptosis. When they induced apoptosis in a p53-independent manner (using anti-Fas antibodies), the cleavage was no longer seen, suggesting that it requires the p53 pathway. As one characteristic of this pathway is the transcription of p53 target genes, the authors then asked whether de novo protein synthesis is needed for the cleavage. Treatment with cycloheximide blocked the cleavage, consistent with this idea.

Cleavage of CIAP1 was not blocked by caspase inhibitors, which means that the cleavage is independent of caspases and is a cause rather than an effect of the cell-death pathway. So might another type of protease be involved in the cleavage? Serine proteases have previously been implicated in apoptosis, so Levine and co-workers treated HeLa cells with etoposide and a general serine protease inhibitor, and

CHROMATIN

Dynamic repression

Heterochromatin protein 1 (HP1) is generally considered as the ‘keeper’ of heterochromatin — transcriptionally silenced, condensed DNA that is also typically associated with the centromeric regions. It is thought to do this by crosslinking chromatin, thereby creating a dense chromatin environment that is impermeable to transcriptional activators.

Two papers, published in Science, from teams led by Richard Festenstein and Tom Misteli, now challenge this view.

Both groups studied the dynamics of HP1 — a major component of heterochromatin — using fluorescence recovery after bleaching (FRAP) on cells that express green fluorescent protein (GFP)–HP1 isoform α, β or γ fusion proteins. Misteli and colleagues stably transfected immortalized Chinese hamster ovary (CHO) cells, whereas the Festenstein group generated transgenic mice that expressed GFP–HP1β isoform protein specifically in T cells.

In CHO cells, fluorescence recovery was rapid for all HP1 isoforms; complete recovery was reached within 5 s in less dense euchromatin and within ~60 s in heterochromatin. In resting T cells taken from the transgenic mice, recovery was much slower and incomplete; ~70% was recovered in 150–200 s in heterochromatin. HP1 mobility in heterochromatin was reduced compared with euchromatin, which possibly reflects the higher density of HP1-binding sites in heterochromatin. Incomplete recovery in resting T cells indicates the presence of an immobile fraction of HP1β molecules, which is larger in heterochromatin than in euchromatin.

To examine the effects of T-cell activation — which triggers gene activation and cell-cycle induction — on HP1 mobility, Festenstein and colleagues measured FRAP in T cells that were taken from mice and activated ex vivo. GFP–HP1β mobility was significantly increased, both in euchromatin and heterochromatin, compared with unstimulated cells. Moreover, the immobile HP1β fraction in euchromatin was reduced to ~10%. The recovery time in heterochromatin in activated T cells (~50–80 s) was similar to that in CHO cells (~60 s), and the fluorescence recovery was indeed almost complete — as in CHO cells.

So, it seems that HP1 binds transiently to heterochromatin and euchromatin, which leads the authors to conclude that heterochromatin is not inaccessible to other factors, and that the continuous exchange of HP1 allows transcriptional regulators to compete for binding, thereby determining the fate of the heterochromatin region. In addition, the increased mobility of HP1 in immortalized cells and activated T cells allows the heterochromatin to be restructured, which might facilitate cell-cycle entry and transcriptional activation.

Ariane Heinrichs

References and links

found that both CIAP1 cleavage and apoptosis were blocked. They obtained similar results using primary mouse thymocytes — thymocytes with no p53 genes failed to cleave CIAP and failed to undergo apoptosis.

To identify the protease involved, Levine and colleagues went back to the literature. Previous reports had shown that mammalian IAPs can interact with a serine protease called HTRA2/OMI, so the authors did a northern blot analysis of HTRA2 messenger RNA levels in HeLa cells during treatment with etoposide. They observed a seven-fold increase in HTRA2 mRNA levels; a similar increase was also seen when HeLa cells were transfected with a p53 expression vector.

The authors therefore conclude that a parallel pathway to that first mapped out in flies indeed exists in mammals, with the subtle difference that CIAP1 is destroyed by protease-mediated cleavage rather than being targeted by ubiquitin for destruction.

Mitochondria in brown adipocyte tissue (BAT) are larger and more numerous than in other cell types; their inner mitochondrial membrane contains uncoupling protein 1 (UCP1), which diverts energy from ATP synthesis to thermogenesis. Nitric oxide (NO) is known to regulate biological functions in mature brown adipocytes, but, until now, NO’s role in mitochondrial biogenesis has not been studied.

In mammals, mitochondrial biogenesis in BAT, and cardiac and skeletal muscle. Using a cyclic GMP analogue and a guanylate-cyclase inhibitor, they also showed that the biogenesis depends on cGMP. And study of mouse white-fat 3T3-L1 and human monocyteic U937 cell lines revealed that the biogenesis was not restricted to brown adipocytes and their differentiation processes.

To investigate the role of endogenous NO, the authors stably transfected HeLa cells with endothelial nitric oxide synthase (eNOS) — the only isoform that is expressed in brown adipocytes, and 3T3-L1 cells under experimental conditions. Induction of eNOS increased mitochondrial biogenesis, an effect that was abolished by a NOS inhibitor.

Cold exposure triggers PGC-1α expression through activation of β3-adrenergic receptors and increases in intracellular CAMP and Ca2++, all of which stimulate NO production in brown adipocytes. So Nisoli et al. studied BAT functions in wild-type and eNOS−/− mice before and after cold exposure. At both temperatures, histological analysis indicated that eNOS−/− BAT was functionally inactive, and mitochondrial biogenesis was impaired.

When the authors looked at the control of biogenesis in the brain, liver and heart of the knockout mice, they found that deletion of eNOS was enough to reduce the number of mitochondria even in tissues that have a basal level of neuronal, and possibly inducible, NOS expression.

In eNOS−/− mice, oxygen consumption rates — an indicator of metabolic rate — were decreased, indicating that BAT-dependent thermogenesis might be impaired. In genetic models of obesity, defective energy expenditure is involved in increased food intake and body-weight gain; eight-week-old eNOS−/− mice showed similar food consumption but weighed more than wild-type mice. So, the increased body weight of eNOS−/− mice could be accounted for by higher feed efficiency (weight gain/food intake) caused by defective energy expenditure.

So what does this mean? The features shown by eNOS−/− mice — reduced mitochondrial number and energy expenditure, weight gain, insulin resistance and hypertension — are all typical of the so-called metabolic syndrome. Millions of people are estimated to have metabolic syndrome, placing them at an increased risk of developing diabetes and cardiovascular disease. However, if the results reported by Nisoli and colleagues are applicable to humans, then we will have “...clues for the prevention and treatment of this condition”.

**References and links**


**WEB SITE** Center for Study and Research on Obesity: [http://www.unimi.it/ateneo/strutt/centric/centrico/centrob.html](http://www.unimi.it/ateneo/strutt/centric/centrico/centrob.html)
This month’s winning image was submitted by Elena Kiseleva (Institute of Cytology and Genetics, Russia; Paterson Institute for Cancer Research, UK; ekiseleva@picr.man.ac.uk). It shows the nuclear pore complexes and Cajal bodies linked by an extensive actin-dependent filamentous network in Xenopus laevis oocyte nuclei. Purple and pink pseudo-colouring represent the anti-coilin-labelled Cajal bodies and the filamentous network, respectively. The specimen was examined in a field emission scanning electron microscope (DS 130F, Topcon). Bar, 167 nm.

The winner would like to acknowledge the help of Sheona Drummond (sdrummond@picr.man.ac.uk) and Steve Bagley (sbagley@picr.man.ac.uk) (Paterson Institute for Cancer Research, UK), who were also involved in this work.

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